

## Observation of *Helicobacter*-like organisms in gastric mucosa of grey foxes (*Urocyon cinereoargenteus*) and bobcats (*Lynx rufus*)

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### Abstract

Archival specimens of gastric mucosa of 10 raccoons (*Procyon lotor*), 9 porcupines (*Erethizon dorsatum*), 6 grey foxes (*Urocyon cinereoargenteus*), 6 bobcats (*Lynx rufus*), 4 skunks (*Mephitis mephitis*), and 3 black bears (*Ursus americanus*) were microscopically examined for evidence of *Helicobacter*-like organisms. Such organisms were seen in the specimens from the grey foxes and bobcats only. Histochemical stains (modified Steiner and carbol fuchsin methods) revealed long spiral organisms within lumina of gastric glands; however, neither gross nor microscopic lesions were observed. By electron microscopy (EM), the organisms were found to be free in the glandular lumina and were seen occasionally in the cytoplasm of gastric epithelial cells. Morphologically, 2 different phenotypes of spiral organisms were identified by EM. The organisms associated with bobcats appeared to be more tightly coiled than those seen in grey foxes. The presence of *Helicobacter*-like organisms in the gastric mucosa of grey foxes has not previously been described.

### Résumé

Des spécimens archivés de muqueuse gastrique provenant de 10 raton-laveurs (*Procyon lotor*), 9 porc-épic (*Erethizon dorsatum*), 6 renards argentés (*Urocyon cinereoargenteus*), 6 lynx (*Lynx rufus*), 4 moufettes (*Mephitis mephitis*) et 3 ours noirs (*Ursus americanus*) ont été examinés par microscopie pour vérifier la présence d'organismes apparentés à *Helicobacter*. De tels micro-organismes ont été vus seulement à partir des échantillons provenant des renards argentés et des lynx. Les colorations histochimiques (méthode modifiée de Steiner et méthode à la fuschine carboxylique) ont permis de mettre en évidence de longs micro-organismes spiralés à l'intérieur de la lumière des glandes gastriques; toutefois, aucune lésion macroscopique ou microscopique n'a été observée. Lors de l'examen en microscopie électronique (EM), les micro-organismes ont été vus libres dans la lumière glandulaire et en quelques occasions dans le cytoplasme des cellules épithéliales gastriques. Basé sur l'apparence morphologique, deux types phénotypiques différents d'organismes spiralés ont été identifiés par EM. Les micro-organismes associés aux lynx semblaient spiralés de manière plus serrée que ceux observés chez les renards argentés. La présence d'organisme apparenté à *Helicobacter* dans la muqueuse gastrique de renard argenté n'avait pas encore été décrite.

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*Helicobacter* spp. are spiral bacteria considered to be responsible for clinical or subclinical gastrointestinal disease in people and animals (1,2). *Helicobacter*-like organisms have been reported to be present in gastric mucosa of a variety of wild carnivores, including tigers, lions, pumas, cervals, wolves, hyenas (3), red foxes (4), cougar (5), and various small felids (6), but to our knowledge have not previously been reported as occurring in grey foxes. We describe the detection of *Helicobacter*-like organisms in the gastric mucosa of grey foxes and bobcats by light and electron microscopy (EM).

The gastric mucosa of 38 animals from 6 wildlife species was examined microscopically for the presence of *Helicobacter*-like organisms. Included in the study were specimens from 10 raccoons (*Procyon lotor*) (Iowa), 9 porcupines (*Erethizon dorsatum*) (Pennsylvania), 6 grey foxes (*Urocyon cinereoargenteus*) (Ohio), 6 bobcats (*Lynx rufus*) (Arkansas), 4 skunks (*Mephitis mephitis*) (Pennsylvania), and 3 black bears (*Ursus americanus*) (Pennsylvania).

The animals were assessed for the presence of background lesions in a given geographic location or were obtained for experiments evaluating the safety of 2 candidate oral glycoprotein-rabies vaccines for raccoons [vaccinia-rabies (VR-G) and raccoonpox-rabies (RP-G) vaccines] in nontarget species (7,8). The grey foxes and the bobcats were used in simultaneous vaccine studies. They had been live trapped and transported to the Centers for Disease Control and Prevention (CDC) in Atlanta, where they were housed in individual raised stainless steel cages. They had been quarantined for at least 30 d, received routine veterinary care, been fed commercial dog and cat food, respectively, and been provided water ad libitum. The experimental period was 4 wk. A detailed necropsy had been carried out on each carcass and tissue samples collected for virologic and histopathologic study.

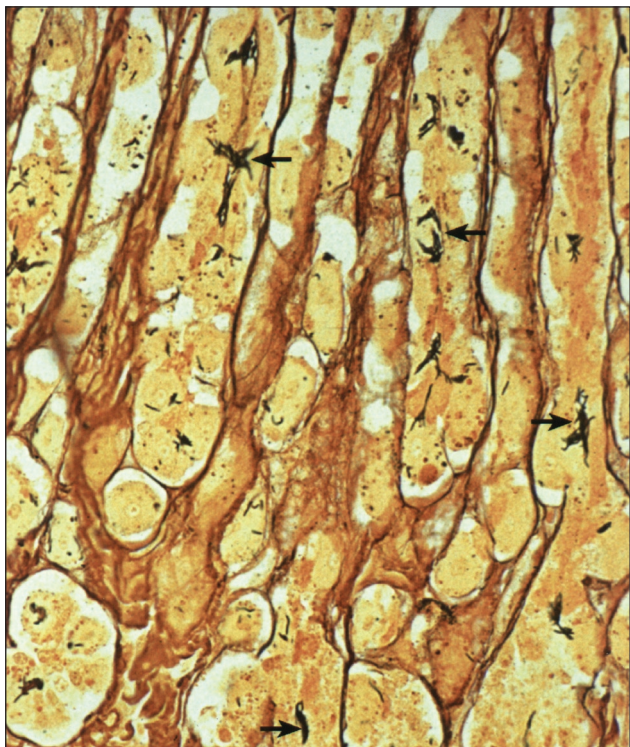
Tissues for histopathologic study had been immersion fixed in 10% neutral buffered formalin, processed for routine histology,

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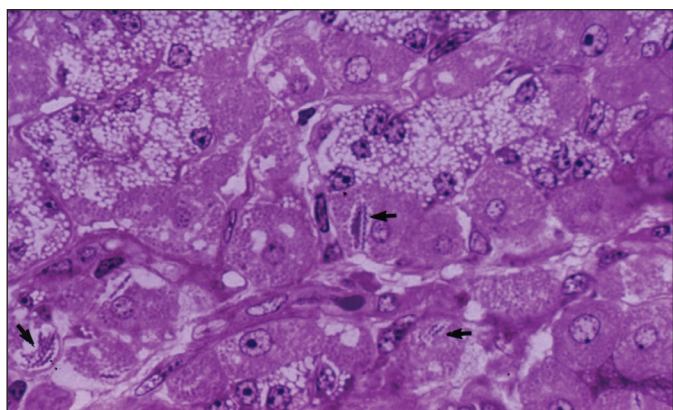
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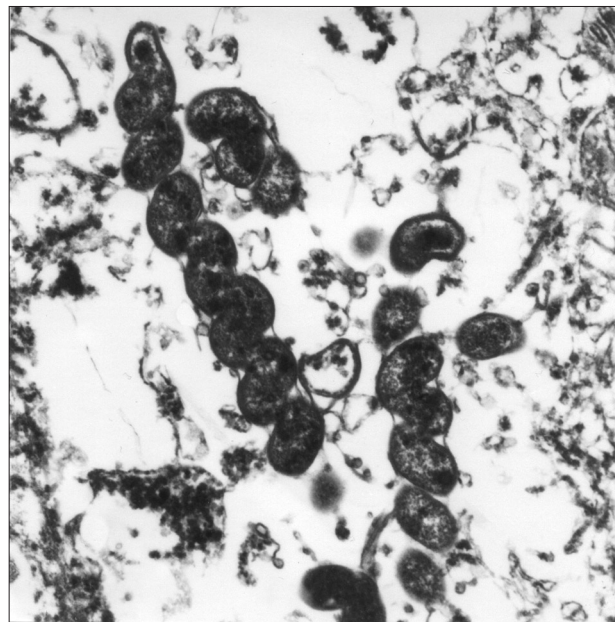


**Figure 1.** Gastric mucosa of a grey fox, showing many silver-positive *Helicobacter*-like organisms (arrows). Modified Steiner stain; X300.

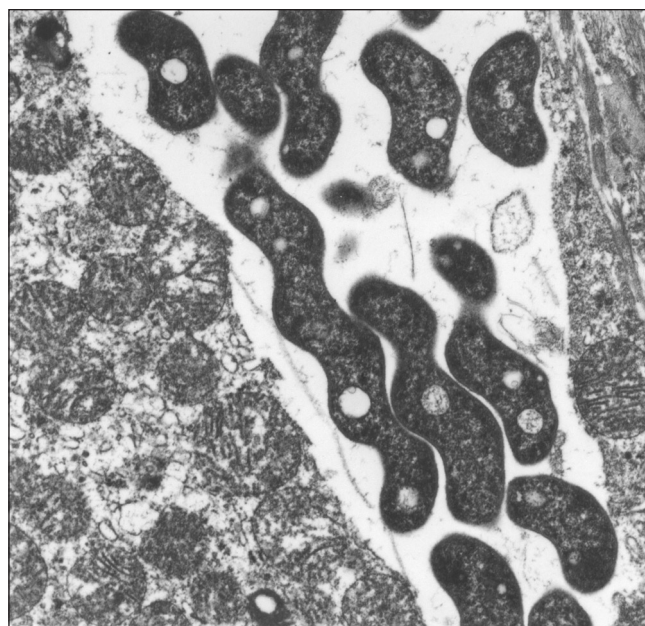


**Figure 2.** Gastric mucosa of a bobcat, showing characteristic spiral organisms resembling *Helicobacter* (arrows). Formalin-fixed tissue; plastic section stained with toluidine blue; X800.

sectioned at 4  $\mu\text{m}$ , and stained with hematoxylin and eosin (HE) for examination by light microscopy. For this study, only the relevant microscopic findings in gastric mucosa were documented. Histologic sections of the stomach were also stained by the modified Steiner method (9) and a carbol fuchsin method (10). Selected formalin-fixed gastric mucosa in which *Helicobacter*-like organisms were detected was placed in 2.5% glutaraldehyde in cacodylate buffer, then in 1.0% osmium tetroxide in cacodylate buffer, and embedded in Epon-araldite mixture. Thick sections (1  $\mu\text{m}$ ) were stained with toluidine blue. Selected areas with the organisms were thin-sectioned, stained with saturated uranyl acetate and lead citrate, and



**Figure 3.** Electron micrograph of gastric mucosa of a bobcat, showing spiral, *Helicobacter*-like organisms. Formalin-fixed tissue stained with uranyl acetate and lead citrate; X11 830.



**Figure 4.** Electron micrograph of gastric mucosa of a grey fox, showing spiral, *Helicobacter*-like organisms. Formalin-fixed tissue stained with uranyl acetate and lead citrate; X11 830.

examined with an electron microscope (Philips model 410; FEI Company, Hillsboro, Oregon, USA).

Gross lesions were not observed in the stomach of any of the animals. Light microscopic examination of sections stained with HE and by the modified Steiner and carbol fuchsin methods revealed spiral organisms within lumina of gastric glands of all the grey foxes (Figure 1) and bobcats (Figure 2) but none of the other animals. The modified Steiner method was considered to be better than the carbol



fuchsin method for detecting the organisms. The characteristic spiral morphology was difficult to demonstrate by light microscopy (Figure 1) but was obvious in tissues processed for EM (Figures 2 to 4). By EM, the organisms were seen to be free in the glandular lumina and, as well, were occasionally observed in the cytoplasm of gastric epithelial cells (Figure 2). Morphologically, different phenotypes of spiral organisms were identified by EM in the grey foxes and the bobcats. In both species, the organisms were similar in thickness; however, the organisms appeared to be more tightly coiled in the bobcats (Figure 3) than in the grey foxes (Figure 4).

Although *Helicobacter*-like organisms have been observed in gastric mucosa of a variety of wild carnivores (3–6), they have not previously been described in gastric mucosa of grey foxes. In some host species (ferrets and humans), lesions associated with the presence of *Helicobacter*-like organisms range from mild gastritis to gastric neoplasia (1,2,11). However, neither gross nor microscopic lesions associated with the presence of *Helicobacter*-like organisms were seen in either the grey foxes or the bobcats examined. The absence of gastric lesions in bobcats is consistent with an earlier report on the occurrence of *Helicobacter*-like organisms in bobcats (6). These findings suggest that the gastric spiral organisms are either commensals or opportunistic pathogens (6).

Since the *Helicobacter*-like organisms were present in all the grey foxes and bobcats, it could be speculated that the animals acquired the infection from the environment or from infected individuals while housed in laboratory facilities. However, since the animals were housed in individual cages during the experimental period, their chances of acquiring infection at that time were low and, therefore, it is most likely that they were infected before their housing at CDC. In a related species, the red fox (*Vulpes vulpes*), there is evidence from Germany that the prevalence of *Helicobacter*-like organisms can be higher in free-ranging animals than in those confined to farms (88% versus 58%) (4).

In our study, phenotypic differences were observed between the *Helicobacter*-like organisms seen in gastric mucosa of grey foxes and bobcats. This suggests that 2 morphologically different species of organisms existed in the experimental animals. Future investigation of *Helicobacter*-like infections in these species should include examination of gastric mucosa of free-ranging animals for the presence of spiral organisms and, if such organisms are present, should be directed at identifying the species that colonize gastric mucosa.

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